

IN THE CLAIMS

1 1. (amended herein) A method for making an infectious adenovirus having enhanced efficiency  
2 which comprises contacting a cell with or introducing into a cell:

3 (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
4 of intermolecular recombination, are [insufficient]incapable to encode an infectious,  
5 replicable or packageable adenovirus; and

6 (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence  
7 of adenoviral replication factors provided in trans or intermolecular recombination  
8 with said first nucleic acid sequence, are [insufficient]incapable to encode an  
9 infectious, replicable or packageable adenovirus;

10 provided that said first and said second nucleic acid sequences each comprise a head-to-head  
11 ITR junction and said first nucleic acid and said second nucleic acid comprise recombinase  
12 recognition sites and wherein said first and said second nucleic acids are contacted with a  
13 recombinase which recognizes said first nucleic acid and said second nucleic acid  
14 recombinase recognition sites; whereby said first and said second nucleic acids recombine  
15 to form said infectious adenovirus.

1 2 (original) The method according to claim 1 wherein said first nucleic acid sequence is a  
2 plasmid containing a circularized adenovirus DNA molecule.

1 3 (amended herein) The method according to claim 2 wherein said plasmid includes a bacterial  
2 origin of DNA replication, an antibiotic resistance gene for selection in bacteria, a deletion  
3 or modification in E1 that renders the adenoviral sequences [insufficient]incapable to form  
4 infectious virus, or an expression cassette encoding a site-specific recombinase, and  
5 combinations thereof.

1 4 (original) The method according to claim 2 wherein said adenovirus DNA has a deletion of

1 an adenoviral packaging signal, or wherein said packaging signal is flanked on either side  
by at least one site-specific recombinase recognition site.

1 5 (original) The method according to claim 4 wherein said adenovirus DNA comprises (i) a  
2 deletion of, (ii) a modification in, or (iii) sequences flanked with a site-specific recombinase  
3 recognition site, of an adenoviral gene selected from the group consisting of adenoviral E1  
4 sequences extending beyond said packaging signal, adenoviral fibre gene sequences,  
5 adenoviral E3 gene sequences, adenoviral E4 gene sequences, and combinations thereof.

1 6 (original) The method according to claim 5 wherein said adenovirus DNA has a *lox* site  
2 located 5' of a pIX gene.

1 7 (original) The method according to claim 2 wherein said plasmid is selected from the group  
2 consisting of pBHGlox $\Delta$ E1,3, pBHG11lox, pBHGdX1Plox, pBHGE3lox, pFG173lox,  
3 pBHGlox $\Delta$ E1,3Cre.

1 8 (original) The method according to claim 1 wherein said second nucleic acid sequence is a  
2 plasmid comprising:

- 3 (a) said head-to-head ITR junction, and a packaging signal contained within the leftmost  
4 approximately 350 nt of the adenovirus genome;  
5 (b) a polycloning site or a foreign DNA or an expression cassette; and optionally,  
6 (c) a *lox* P site 3' of said polycloning site, foreign DNA, or expression cassette.

1 9. (amended herein) The method according to claim 8 wherein said plasmid is selected from  
2 the group consisting of p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ ,  
3 pMH4lox, pMH4lox $\Delta$ , pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ ,  
4 pCA36lox, pCA36lox $\Delta$ , pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pCA35lox,  
5 pCA35lox $\Delta$ CreITR, pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, and

6 pDC118[, which, as optionally needed, undergo additional modification to provide a head-to-  
7 head ITR junction].

1 10. (amended herein) A recombinant adenovirus vector system comprising:

2 (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
3 of intermolecular recombination, are [insufficient]incapable to encode an infectious,  
4 replicable or packageable adenovirus, said first nucleic acid sequence comprising a  
5 head-to-head ITR junction and at least one site-specific recombinase recognition  
6 target site which is recognized by a site-specific recombinase; and,

7 (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence  
8 of adenoviral replication factors provided in trans or intermolecular recombination  
9 with said first nucleic acid sequence, are [insufficient]incapable to encode an  
10 infectious, replicable or packageable adenovirus, said second nucleic acid sequence  
11 comprising a head-to-head ITR junction and a site-specific recombinase recognition  
12 target site sufficiently identical with said recombinase recognition target site in said  
13 first nucleic acid as to be recognized by the same site-specific recombinase which  
14 recognizes said site-specific recombinase recognition target site in said first nucleic  
15 acid;

16 wherein said first and said second nucleic acid sequences, in combination and following site-  
17 specific intermolecular recombination, result in production of an infectious adenovirus, and  
18 wherein a site-specific recombinase which recognizes said site-specific recombinase  
19 recognition target sites either (i) is expressed by a cell into which said first and said second  
20 nucleic acids are introduced, (ii) is operatively encoded by said first nucleic acid, said second  
21 nucleic acid or both, or (iii) is provided in trans through expression from a third nucleic acid,  
22 or (iv) is provided in trans as an active protein.

1 11. (amended herein) The recombinant adenovirus vector system of claim 10 comprising:

2 (a) a first [plasmid]nucleic acid sequence comprising a plasmid selected from the group

- 3 consisting of pBHGlox $\Delta$ E1,3, pBHG11lox, pBHGlox $\Delta$ E1,3Cre, and  
4 pBHGlox $\Delta$ E1,3CreR, containing a circularized adenovirus DNA molecule and  
5 optionally including a bacterial origin of DNA replication and an antibiotic resistance  
6 gene for selection in bacteria and having a deletion or modification of the packaging  
7 signal, of additional E1 sequences, of E3, E4 or fibre, wherein said site-specific  
8 recombinase recognition target site is a *lox P* site located adjacent the pIX gene, E3,  
9 E4 or fibre of the virus, said plasmid optionally encoding Cre recombinase;
- 10 (b) a second [plasmid]nucleic acid sequence comprising a plasmid selected from the  
11 group consisting of p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ ,  
12 pMH4lox, pMH4lox $\Delta$ , pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox,  
13 pCA14lox $\Delta$ , pCA36lox, pCA36lox $\Delta$ , pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT,  
14 pCA35lox, pCA35lox $\Delta$ CreITR, pDC111, pDC112, pDC113, pDC114, pDC115,  
15 pDC116, pDC117, pDC118, and identifiable combinations thereof[, and which, as  
16 optionally needed, undergo additional modification to provide a head-to-head ITR  
17 junction], and comprising:
- 18 (i) all or most of the left ITR and the packaging signal contained within the  
19 leftmost approximately 350 nt of the Ad genome or a head-to-head ITR  
20 junction;
- 21 (ii) a polycloning site or a foreign DNA or an expression cassette; and,  
22 (iii) as said site-specific recombinase recognition target site, a *lox P* site 3' of said  
23 polycloning site or foreign DNA or expression cassette; and
- 24 (c) a cell line that is normally able to support replication of adenovirus and which  
25 optionally expresses the recombinase Cre that is able to catalyse site-specific  
26 recombination between said *lox P* sites.

- 1 12. (original) The recombinant adenovirus vector system of claim 10 wherein said cell further  
2 expresses adenoviral E1.

1 13. (amended herein) The recombinant adenovirus vector system of claim 10 wherein said first  
2 [plasmid]nucleic acid sequence and said second [plasmid]nucleic acid sequence are  
3 cotransfected into said cell to produce an infectious virus vector comprising a left end, a  
4 polycloning site[,]or a foreign DNA[,] or an expression cassette [derived] from said second  
5 [plasmid]nucleic acid sequence, joined to [the]a remaining portion of the [viral  
6 DNA]adenoviral sequences [derived] from said first [plasmid]nucleic acid sequence.

1 14. (Amended herein) The recombinant adenovirus vector system of claim 10 wherein said cell  
2 is co-transfected with a first [DNA]nucleic acid sequence from a virus selected from the  
3 group consisting of AdLC8, AdLC8cluc, AdLC8cCE199, comprising a packaging signal  
4 flanked by *loxP* sites, and a second [DNA]nucleic acid sequence comprising a packaging  
5 signal wherein said second [DNA]nucleic acid sequence is selected from the group  
6 consisting of p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ , pMH4lox,  
7 pMH4lox $\Delta$ , pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ , pCA36lox,  
8 pCA36lox $\Delta$ , pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pCA35lox, pCA35lox $\Delta$ CreITR, pDC111,  
9 pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, pDC118, and identifiable  
10 combinations thereof[ which as needed undergo modification to provide a head-to-head ITR  
11 junction], whereby said *loxP* sites flanking said packaging signal of said first [DNA]nucleic  
12 acid sequence are acted upon by Cre recombinase expressed in said cells to induce excision  
13 of said packaging signal, producing a noninfectious virus genome incapable of packaging its  
14 DNA into virions unless joined by Cre-mediated recombination to the *loxP* site of said  
15 second [DNA]nucleic acid sequence to reconstitute a packaging signal therein.

1 15. (amended herein) The recombinant adenovirus vector system of claim 14 wherein, prior to  
2 said co-transfection, said first [DNA]nucleic acid sequence is cleaved with a restriction  
3 enzyme that cuts between said *loxP* sites.

1 16. (Amended herein) A kit for construction of recombinant adenovirus vectors comprising:

- 2 (A) a first nucleic acid sequence encoding adenovirus sequences which, in the absence  
3 of intermolecular recombination, are [insufficient]incapable to encode an infectious,  
4 replicable or packageable adenovirus, said first nucleic acid sequence comprising a  
5 head-to-head ITR junction and at least one site-specific recombinase recognition  
6 target site which is recognized by a site-specific recombinase;
- 7 (B) a second nucleic acid sequence encoding adenovirus sequences which, in the absence  
8 of adenoviral replication factors provided in trans or intermolecular recombination  
9 with said first nucleic acid sequence, are [insufficient]incapable to encode an  
10 infectious, replicable or packageable adenovirus, said second nucleic acid sequence  
11 comprising a head-to-head ITR junction and a site-specific recombinase recognition  
12 target site sufficiently identical with said recombinase recognition target site in said  
13 first nucleic acid as to be recognized by the same site-specific recombinase which  
14 recognizes said site-specific recombinase recognition target site in said first nucleic  
15 acid; and
- 16 (C) a cell wherein, when said component (a) and said component (b) are cotransfected  
17 and recombined through the action of a recombinase which recognizes said  
18 recombinase recognition sites, an infectious recombinant adenovirus vector is  
19 produced.

1 17 (original) The kit according to claim 16 wherein component (a) is selected from the group  
2 consisting of pBHGlox $\Delta$ E1,3, pBHG11lox, pBHGdX1Plox, pBHGE3lox, and  
3 pBHGlox $\Delta$ E1,3Cre.

1 18 (amended herein) The kit according to claim 16 wherein said component (b) is selected from  
2 the group consisting of p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ ,  
3 pMH4lox, pMH4lox $\Delta$ , pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ ,  
4 pCA36lox, pCA36lox $\Delta$ , pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pCA35lox,  
5 pCA35lox $\Delta$ CreITR, pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117,

6 pDC118, and identifiable combinations thereof[, which, as optionally needed, undergo  
7 additional modification to provide a head-to-head ITR junction].

1 19 (original) The kit according to claim 16 wherein said cell of (c) is selected from the group  
2 consisting of 293 cells, 293 cells expressing Cre, PER-C6 cells expressing Cre, 911 cells  
3 expressing Cre, and wherein said recombinase recognition sites are *lox P* sites.

1 20 (original) The recombinant adenovirus vector system according to claim 10 wherein an  
2 adenoviral gene mutation is rescued into said adenoviral vector recombinant.

1 21 (original) The recombinant adenovirus vector system according to claim 20 wherein said  
2 adenoviral gene mutation rescued into said adenoviral vector recombinant is a mutation in  
3 the adenoviral fibre gene, the adenoviral E4 gene, the adenoviral E3 gene, or combinations  
4 thereof.

1 22 (original) The recombinant adenovirus vector system according to claim 10 wherein said first  
2 nucleic acid sequence comprises a recombinase recognition site and a deletion in the  
3 adenoviral fibre gene.

1 23 (original) The recombinant adenovirus vector system of claim 10 comprising:  
2 (a) a first adenovirus vector having a fibre gene flanked by *loxP* sites;  
3 (b) a plasmid comprising a bacterial origin of replication, a bacterial antibiotic resistance  
4 marker, the right end of the Ad genome encompassing a fibre gene comprising a deletion,  
5 a single *loxP* site located to the left of the fibre gene, and a foreign DNA insert between the  
6 *loxP* site and the fibre gene.

1 24 (original) An adenoviral vector selected from the group consisting of pBHGlox $\Delta$ E1,3,  
2 pBHG11lox, pBHGdX1Plox, pBHGE3lox, pFG173lox, and pBHGlox $\Delta$ E1,3Cre.

1 25 (amended herein) An adenoviral vector selected from the group consisting of p $\Delta$ E1sp1Alox,  
2 p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ , pMH4lox, pMH4lox $\Delta$ , pMH4lox $\Delta$ link,  
3 pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ , pCA36lox, pCA36lox $\Delta$ ,  
4 pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pFG23dX1lox, pAB14lox $\Delta$ , pAB14flox,  
5 pCA35lox $\Delta$ CreITR, and derivatives thereof[, which, as optionally needed, undergo  
6 additional modification to provide a head-to-head ITR junction].

1 26 (original) A cell comprising the adenoviral vector of claim 24.

1 27 (original) A cell comprising the adenoviral vector of claim 25.

1 28 (amended herein) A cell into which has been introduced a first vector selected from the group  
2 consisting of pBHGlox $\Delta$ E1,3, pBHG11lox, pBHGdX1Plox, pBHGE3lox, pFG173lox, and  
3 pBHGlox $\Delta$ E1,3Cre, and a second vector selected from the group consisting of  
4 p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ , pMH4lox, pMH4lox $\Delta$ ,  
5 pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ , pCA36lox, pCA36lox $\Delta$ ,  
6 pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pCA35lox, pCA35lox $\Delta$ CreITR, pDC111, pDC112,  
7 pDC113, pDC114, pDC115, pDC116, pDC117, pDC118, and identifiable combinations  
8 thereof[, which, as optionally needed, undergo additional modification to provide a head-to-  
9 head ITR junction].

1 29 (cancelled)

1 30. (cancelled)

1 31. (amended herein) A composition comprising the recombination product of a first vector  
2 selected from the group consisting of pBHGlox $\Delta$ E1,3, pBHG11lox, pBHGdX1Plox,



pBHGE3lox, pFG173lox, pBHGlox $\Delta$ E1,3Cre, and a second vector selected from the group consisting of p $\Delta$ E1sp1Alox, p $\Delta$ E1sp1Alox $\Delta$ , p $\Delta$ E1sp1Blox, p $\Delta$ E1sp1Blox $\Delta$ , pMH4lox, pMH4lox $\Delta$ , pMH4lox $\Delta$ link, pCA13lox, pCA13lox $\Delta$ , pCA14lox, pCA14lox $\Delta$ , pCA36lox, pCA36lox $\Delta$ , pCA36lox $\Delta$ CreR, pCA36lox $\Delta$ CreT, pCA35lox, pCA35lox $\Delta$ CreITR, pDC111, pDC112, pDC113, pDC114, pDC115, pDC116, pDC117, pDC118, and identifiable combinations thereof[, which, as optionally needed, undergo additional modification to provide a head-to-head ITR junction], wherein said first vector and said second vector are contacted optionally in the presence of Cre recombinase.

32. (original) The composition according to claim 31 wherein said first and said second vectors are contacted inside a cell and said recombination product is harvested from said cell.

33. (amended herein) An improved adenovirus vector system comprising two plasmids, neither of which alone comprises [sufficient] adenoviral sequences capable to produce infectious adenovirus when introduced into a cell but which, when both plasmids are introduced into a cell, recombine to form an infectious recombinant adenovirus, the improvement comprising: (a) inclusion of a head-to-head ITR junction in each of said two plasmids, and (b) inclusion, either in said first plasmid, said second plasmid, in both said first and said second plasmids or into a cell into which said first and said second plasmids are introduced, [sufficient] sequences to encode an active site-specific recombinase, and inclusion in said first and said second plasmid of recombinase recognition sequences, such that upon contact of said first and said second plasmids with said site-specific recombinase, site-specific recombination between said recombinase recognition sequences in said first plasmid and said second plasmid occurs.

34. (amended herein) A two-plasmid system for making an infectious adenoviral vector wherein each plasmid alone comprises [insufficient] adenoviral sequences incapable to encode an infectious adenoviral vector wherein, upon recombination, an infectious adenoviral vector

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4 is produced, provided that each plasmid of said two-plasmid system comprises (a) a head-to-  
5 head ITR junction; and (b) a recombinase recognition site such that upon contact of both  
6 plasmids of said two-plasmid system with a site-specific recombinase, site-specific  
7 recombination between the plasmids of said two-plasmid system occurs.